and a sugar alcohol**[, wherein the carbohydrate is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic, uronic and aldaric acids, disaccharides and polysaccharides]**.

16. (Amended) The method **[for preserving a cell or tissue specimen as claimed in]** of claim 1, wherein **[the contacting step]** equilibrating and thereby dehydrating the specimen is performed at room temperature or higher.

Please cancel Claims 2, 3, 11, 17-24.

Please add new Claims 25 and 26.

25. The method of claim 1, wherein the non-permeating co-solute is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic acid, uronic acid, aldaric acid, a disaccharide and a polysaccharide.

26. The method of claim 12, wherein the non-permeating rehydration co-solute is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic acid, uronic acid, aldaric acid, a disaccharide and a polysaccharide.

REMARKS

In response to the Examiner's comments regarding the IDS, a supplemental IDS will be filed with complete citations for reference numbers 1, 4, 5 and 7.

In response to the Examiner's objection to the specification for lacking an abstract printed on a separate sheet of paper, Applicant has added an abstract on a separate sheet of paper at the end of specification.

In response to the Examiner's suggestion that the specification be amended to make reference to the PCT application and earlier filed provisional application, Applicant has added a first sentence to the specification stating that "[t]his is a 35 U.S.C. §371 application of PCT/US97/15611, filed on September 5, 1997, which claims priority to U.S. Provisional application No. 60/025,570 filed on September 6, 1996."

Rejections under 35 U.S.C. §112, second paragraph

The Examiner indicated that the phrase "characterized by," which appeared in claims 1 and 12, is indefinite. These claims have been amended to delete the phrase "characterized by." In place of the phrase, "characterized by its ability to limit the amount of a permeating

cryoprotectant to permeate into the specimen," Applicant has amended these claims to recite "wherein the non-permeating co-solute effectively decreases the chemical potential of the permeating cryoprotectant thereby limiting the amount of the permeating cryoprotectant which permeates into the specimen." The new phrase more distinctly claims this characteristic of the non-permeating co-solute. No new matter is added by this amendment. Support for the new language can be found throughout the specification. See e.g., pg. 9, lines 31-37, stating that "a non-permeating co-solute... effectively decrease[s] the chemical potential of penetrating cryoprotectants... [thus] the co-solutes will limit the amount of permeating cryoprotectants that move inside cells..." Accordingly, Applicant respectfully requests withdrawal of this §112, second paragraph rejection.

The Examiner also indicated that Claim 3 was indefinite for repeating the first component of the cryoprotectant solution. Claim 3 has been canceled. Thus, this rejection is now deemed moot.

The Examiner also indicated that Claim 4 was indefinite for failing to state whether the components are intended to be penetrating or non-penetrating cryoprotectants. Claim 4 has been canceled, so this rejection is now deemed moot.

The Examiner also indicated that the Markush groups in Claims 6, 15, 21 and 24 were improper because more than one "and" appeared. These claims have been amended so that only one "and" appears in each Markush group. Accordingly, Applicant respectfully requests withdrawal of this §112, second paragraph rejection.

The Examiner also stated that the phrase "does not substantially damage cells" in Claim 7 was indefinite. Claim 7 has been amended to delete the phrase. Amended Claim 7 now recites "[t]he method of claim 1, wherein the total concentration of non-permeating co-solute is between about 0.1 and 0.7 mol/l." Thus, Applicant respectfully requests withdrawal of this §112, second paragraph rejection.

The Examiner also indicated that "the rehydration solution" recited in Claim 11 lacked antecedent basis. Claim 11 has been canceled. Thus, this rejection is now deemed moot.

The Examiner also indicated that Claim 13 was indefinite because it was unclear whether the permeating cryoprotectants were components of the rehydration solution or merely the same permeating cryoprotectants from the first steps. Applicant amended the claim to clarify that the rehydration solution "further compris[ed] a permeating rehydration cryoprotectant selected from

the group consisting of dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol." Accordingly, Applicant respectfully requests withdrawal of this §112, second paragraph rejection.

The Examiner also indicated that the phrase "stably stored" in Claim 17 was indefinite. Claim 17 has been canceled. Thus, this rejection is now deemed moot.

The Examiner also stated that "Ficol" recited in Claim 20 appeared to be a trademark and may have been misspelled. Claim 20 has been amended accordingly to recite "FICOLL". Thus, Applicant respectfully requests withdrawal of this §112 rejection.

Rejections under 35 U.S.C. §102

The Examiner found that Claims 1-8, 10, 16 and 18-24 were anticipated under §102(b) by U.S. Patent No. 4,980,277 to Junnila. Claims 18-24 have been canceled. The remaining claims all depend from amended Claim 1. Junnila discloses methods of ***freezing*** cells in a solution comprising betaine (a non-permeating co-solute), glycerol (a permeating cryoprotectant) and egg yolk (which according to the Examiner contains a variety of proteins and peptides – a non-permeating cryoprotectant). The entire teaching of Junnila is directed to ***freezing cells for storage in liquid nitrogen (-196° C)***. This is a different process than Applicant's method of preservation by equilibrating and thereby dehydrating the specimen in a vitrification solution comprising a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant..., and vitrifying the dehydrated specimen, without freezing, by cooling to a refrigeration or higher storage temperature," as recited in amended Claim 1. The process of freezing involves the formation of ice crystals, which may be damaging to sensitive biological specimens. In contrast, the process of vitrification, as described in the specification, involves "solidification of samples during cooling, without formation of ice crystals (Fahy, G.M. et al., 1984)." Page 1, lines 18-20. Thus, Junnila fails to teach or suggest a preservation method in which a dehydrated specimen is vitrified, without freezing, by cooling to a refrigeration or higher storage temperature.

The amendments to Claim 1 do not present new matter. The combination of a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant are supported throughout the specification and were recited in original Claim 2, now canceled. Equilibrating and thereby dehydrating the specimen is also supported in the specification as filed. See e.g., pg. 11, line 22, stating that "cells should be substantially dehydrated..."; pg. 12 lines 1-

8, stating that "[a]fter dehydration in cryopreservation (vitrification) solution, cells can be stored at a temperature that is lower than the vitrification temperatures... Prior to dehydration, cells may be loaded in a low concentration (5-40 wt%), non-damaging solution of permeating cryoprotectant to protect cells from damage during dehydration..."; pg. 12, lines 27-33, stating that "[t]he invention allows one to... significantly increase... the time of cell equilibration (dehydration) in the vitrification solution...", and pg. 13-14, lines 36-1, stating that "[t]he method of the present invention encompasses dehydration of specimens..." (emphasis added).

With respect to vitrifying the dehydrated specimen (as opposed to freezing the specimen), by cooling to refrigeration or higher storage temperature, support can be found throughout the specification. See e.g., pg. 1, lines 17-20, stating that "[v]itrification is an alternative approach to cryopreservation that utilizes solidification of samples during cooling, without formation of ice crystals;" pg. 12, lines 29-30, stating "... to obtain a stable vitrification of the cells during cooling..."; pg. 13, lines 17-21, stating that "[b]y increasing the intracellular and extracellular vitrification temperatures, one will be able to increase storage temperature to a refrigeration or even room temperature...". In addition, Claim 17 as originally filed, recited that "the specimen can be stably stored at a temperature greater than 4° C."

Junnila teaches a conventional method of cryopreservation by freezing to very low (liquid nitrogen) temperatures. Junnila neither teaches dehydration by equilibration in a vitrification solution or subsequent vitrification, without freezing, by cooling to a refrigerated or higher storage temperature, as recited in amended Claim 1. Accordingly, because Junnila fails to teach or suggest every element of the process recited in amended Claim 1, from which Claims 2-8, 10 and 16 depend, Applicant respectfully requests withdrawal of the §102 rejection of Claims 1-8, 10 and 16 based on Junnila.

The Examiner also stated that Claims 1-7, 16 and 18-24 were rejected under §102(b) as being anticipated by Titterington et al. Claims 18-24 have been canceled. This reference teaches immersion of specimens in liquid nitrogen (-196° C) in the presence of sucrose (a non-permeating co-solute), glycerol (a permeating cryoprotectant) and Percoll (a non-permeating cryoprotectant). Like Junnila, however, the disclosure of Titterington et al. is limited to ***rapid freezing to –196° C***. Titterington et al. does not teach equilibrating and thereby dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant, and then vitrifying the dehydrated specimen, without freezing, by

cooling to a refrigerated or higher storage temperature, as recited in amended Claim 1. Accordingly, Applicant respectfully requests withdrawal of the §102 rejection of Claims 1-7 and 16 based on Titterington et al.

The Examiner also rejected Claims 1-6, 9, 10, 16 and 18-24 as being anticipated by Rall et al. Claims 18-24 have been canceled. Rall et al. discloses progressive or step-wise cooling of embryos down to –196° C (liquid nitrogen) in a vitrification solution comprising 0.00556 mol/l glucose (non-permeating co-solute), glycerol (permeating cryoprotectant) and BSA (non-permeating cryoprotectant). As detailed above with respect to Junnila, the present invention for dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant, and then vitrifying the dehydrated specimen, without freezing, by cooling to a refrigerated or higher storage temperature, is neither taught nor suggested by the cryopreservation in liquid nitrogen (–196° C) disclosed by Rall et al. Moreover, the very low concentration of non-permeating co-solute employed by Rall would not be sufficient to "effectively decrease the chemical potential of the permeating cryoprotectant," as recited in Claim 1. Thus, Applicant respectfully requests withdrawal of the §102 rejection of Claims 1-6, 9, 10 and 16 over Rall et al.

The Examiner also indicated that Claims 1-6, 10, 16 and 18-24 were anticipated under §102(b) by U.S. Patent No. 5,160,313 to Carpenter. This reference teaches methods of thawing transplantable tissues that had been previously cryopreserved with an intracellular cryoprotectant (e.g., DMSO). As the Examiner noted, the specification (Example 1) teaches cryopreservation of heart valve leaflets in DMEM with 10% FCS and 10% DMSO. No detailed preservation parameters are disclosed. Instead, the thrust of the teaching in Carpenter focuses on the parameters for thawing frozen specimens. See e.g., Example 1, col. 4, lines 61, stating that "[t]he segments are then thawed...". Moreover, it is also clear that cryopreservation is used in the '313 patent to mean ***freezing***. See e.g., col. 2, lines 35-36, stating that "...the transplantable tissue is subjected to ***an appropriate freezing protocol***." Carpenter fails to teach or suggest dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant and vitrifying the dehydrated specimen, without freezing, by cooling to a refrigeration or higher storage temperature, as recited in amended Claim 1. Accordingly, Applicant respectfully requests that the Examiner withdraw the §102 rejection of Claims 1-6, 10 and 16, based on Carpenter.

The Examiner also rejected Claims 1, 6, 8-10 and 22-24 under §102(b) as anticipated by U.S. Patent No. 5,217,860 to Fahy. This reference teaches an apparatus and methods for preservation of organs, including a cryoprotectant solution comprising DMSO (permeating cryoprotectant), propanediol or dextrose (non-permeating co-solute) and formamide (another permeating cryoprotectant), the concentrations of which may be increased in a progressive manner. Fahy does not teach use of a non-permeating polymeric cryoprotectant. Moreover, Fahy does not teach dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant and vitrifying the dehydrated specimen, without freezing, by cooling to a refrigerated or higher storage temperature. Indeed, Fahy discloses that after equilibrating the tissue with vitrification solution (by perfusion), "[t]he next step of any practical vitrification procedure will be to remove the organ from the perfusion machine and cool it to cryogenic temperatures, with or without prior pressurization." Col. 27, lines 39-42. Cryogenic preservation is defined by Fahy as "preservation at very low temperatures." Col. 1, lines 16-17. Claim 1 was amended to recite equilibrating and thereby dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant and vitrifying the dehydrated specimen, without freezing, by cooling to a refrigerated or higher storage temperature. Since Fahy fails to teach or suggest all of the recited elements, Applicant respectfully requests withdrawal of the §102(b) anticipation rejection of Claims 1, 6 and 8-10.

The Examiner also rejected Claims 1-8, 11, 12, 15, 16 and 18-24 under §102(b) as anticipated by WO 94/13135. This PCT publication teaches cryopreservation by freezing epithelial sheets at a temperature in the range of –20° C to –80° C in a cryopreservation solution comprising glycerol (permeating cryoprotectant), 0.004 mol/l glutamine (non-permeating co-solute) and PEG (non-permeating cryoprotectant). This reference, like the previous ones, is directed at improved methods of freezing biological specimens. The disclosed cryopreservation solutions are formulated to minimize the cellular "volume increase" and "macrocrystal formation" during freezing. This reference fails to teach dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant and vitrifying the dehydrated specimen, without freezing, by cooling to a refrigerated or higher storage temperature, as recited in amended Claim 1. Furthermore, the concentration (0.004 mol/l glutamine (non-permeating co-solute) is far to low, in relation to the disclosed 15% glycerol

(permeating protectant), to "effectively decrease the chemical potential of the permeating cryoprotectant thereby limiting the amount of the permeating cryoprotectant which permeates into the specimen," as recited in amended Claim 1. Thus, Applicant respectfully requests that the Examiner withdraw this §102(b) rejection of Claims 1-8, 12, 15 and 16 based on WO 94/13135.

The Examiner also rejected Claims 1-7, 9, 11-16 and 18-23 as anticipated by U.S. Patent No. 5,364,756 to Livesey. This patent teaches a variety of cryopreservation solutions, some comprising DMSO and propylene glycol (permeating cryoprotectants), raffinose, trehalose, sucrose and proline (non-permeating co-solutes) and PVP and dextran (non-permeating cryoprotectants). Livesey also teaches cryopreservation by first rapidly freezing (preferably by nebulization) the specimen to a vitrified state, followed by drying the frozen specimen by sublimation. "[R]egardless of the form of cooling that is used, it is believed that amorphous phase water [–160° C transition pt.], cubic ice crystals [–130° C transition pt.] and hexagonal ice crystals [–120° C transition pt.] are present in the final product." Col. 14, lines 16-19. Next, "the sample must be loaded into the dryer without temperature elevation above its lowest transition temperature... Ideally, loading occurs under liquid nitrogen at –190° C, well below the lowest discernible transition of –160° C for pure, ultrarapidly-cooled amorphous water. If however, the sample is predominantly cubic ice or a mixture of water and cryoprotectants with a glass transition temperature of the order of –100° C to –130° C, a closed circuit refrigeration system may be sufficient to enable maintenance of the sample temperature below the onset of transition." Col. 15, lines 34-45. Clearly, the method of cryopreservation disclosed by Livesey involved freezing the specimen at very low temperatures prior to dehydration. Subsequently, the frozen specimen is dehydrated by molecular distillation (sublimation) drying with gradual increases in temperature after each type of ice has been sublimated. Final storage temperatures in the range of -20° C to 20° C are disclosed; however, these storage temperatures are reached by heating rather than cooling.

In the earlier referenced Livesey patent, U.S. Patent No. 4,865,871, cited for the method of drying erythrocytes (Example 4 of the '756 patent), the Patentee states that "[t]he drying process begins at approximately –140° C and is completed before ***devitrification*** at –80° C. The sample is then heated from –70° C to +25° C in 4 hours." Col. 11, lines 13-16. Consequently, Livesey (5,364,756 and 4,865,871) fail to disclose dehydrating a specimen and then vitrifying the dehydrated specimen, without freezing, by cooling to a refrigeration or higher storage

temperature, as recited in amended Claim 1. Therefore, Applicant respectfully requests withdrawal of the Examiners rejection of Claims 1-7, 9 and 11-16 as anticipated under §102(b) by Livesey (5,364,756).

The Examiner also rejected Claims 1-7 and 9-24 under §102(e) as anticipated by U.S. Patent No. 5,800,978 to Goodrich. As noted by the Examiner, Goodrich discloses numerous cryopreservation solutions comprising permeating cryoprotectants, non-permeating co-solutes and non-permeating cryoprotectants. Goodrich also teaches freezing cells in these cryopreservation solutions, and in some cases (See e.g., Example 6), subsequent dehydration by sublimation, similar to Livesey. Goodrich does not disclose dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant and vitrifying the dehydrated specimen, without freezing, by cooling to a refrigeration or higher storage temperature, as recited in amended Claim 1. Thus, Applicant respectfully requests that the Examiner withdraw this §102(e) rejection of Claims 1-7 and 9-16 based on Goodrich (5,800,978).

Rejections under 35 U.S.C. §103

The Examiner rejected Claims 1-24 as obvious under §103 over U.S. Patent No. 5,364,756 (Livesey) in view of U.S. Patent No. 5,217,860 (Fahy) taken with U.S. Patent No. 4,865,871 (Livesey) or U.S. Patent No. 5,879,876 (Wolfinbarger). Applicant respectfully asserts that the method of equilibrating and thereby dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant and vitrifying the dehydrated specimen, without freezing, by cooling to a refrigeration or higher storage temperature, as recited in amended Claim 1, is very different from and non-obvious over the combination of cited references, because these references ALL teach methods of protecting cells or tissues from ice formation which occurs during freezing at very low temperatures. Thus, whereas various protectant formulations comprising permeating protectants, non-permeating co-solutes and non-permeating protectants have been disclosed in the cited art for minimizing damage caused by ice crystallization during freezing, none of the references teach or even suggest the use of such formulations for vitrification of dehydrated specimens (i.e., solidification of specimens without formation of ice crystals) by cooling at a refrigeration or higher storage temperature, as recited in amended Claim 1.

The primary reference, the 5,364,756 patent (plus the earlier filed 4,865,871 patent) to Livesey, disclose in great detail, the different forms of ice crystals which form during practice of their method during rapid freezing at very low temperatures. These patents teach that vitreous water must be sublimated from each form of ice at temperatures below the respective transition points. The combined disclosures teach against dehydration prior to vitrification. Indeed, the '871 patent explicitly indicates that the specimens are ***vitrified*** by cooling to –190° C or below and subsequently ***devitrified*** during dehydration as the temperature increases up to –80° C. Col. 11, lines 13-15. The other references cited by the Examiner deal with increasing and decreasing the concentrations of cryoprotectants during freezing and reconstituting steps, respectively. The additional references cited in combination with the primary reference, however, do not cure the defect in the prior art, in failing to teach or suggest preservation by dehydrating a specimen and then vitrifying the dehydrated specimen, without freezing, by cooling to a refrigeration or higher storage temperature, as recited in amended Claim 1, since all of these references teach cryogenic (very low temperature) preservation. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw the obviousness rejection of all pending claims.

CONCLUSIONS

In view of the foregoing amendments and remarks, the present application is submitted as in condition for allowance, and such action is earnestly solicited. If any matters should remain, the Examiner is invited to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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Dated: 9/28/00

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